# Reprogramming the Mycobacterium tuberculosis transcriptome during pathogenesis

Article (Accepted Version)

# Reprogramming the *Mycobacterium tuberculosis* transcriptome during pathogenesis

Simon J Waddell

Brighton and Sussex Medical School, University of Sussex, Brighton, BN1 9PX

Tel: +44 (0)1273 877572

Fax: +44 (0)1273 877576

Email: s.waddell@bsms.ac.uk

## Abstract

Transcriptional profiling has revealed that *Mycobacterium tuberculosis* adapts both its metabolic and respiratory states during infection, utilising lipids as a carbon source and switching to alternative electron acceptors. These global gene expression datasets may be exploited to identify virulence determinants and to screen for new targets for rational drug design. Characterising the changing physiological predicament of distinct *M.tb* populations during infection will help expose the fundamental biology of *M.tb*; highlighting mechanisms that influence tuberculosis pathogenicity.

## Introduction

*Mycobacterium tuberculosis* (*M.tb*) is able to survive in multiple diverse surroundings during the infectious process; from intracellular environments permissive and non-permissive for growth, to the extracellular milieu of heterogeneous lung lesions, to transmission by aerosol and the establishment of a new infection. Transcriptional profiling provides a global method for describing and modelling the adaptations necessary for *M.tb* survival in each scenario. The sequencing of the *M.tuberculosis* H37Rv genome [1] together with the development of mycobacterial RNA isolation [2-4] and amplification [5] techniques has enabled genome-wide microarray profiling to be applied to tissue culture, animal model or natural *M.tb* infection settings. These intracellular and *in vivo*-derived *M.tb* transcriptional signatures, relative in most cases to log phase bacilli grown in axenic culture, capture key metabolic modifications and immune mediator expression that ultimately results in TB disease. This review focuses on the remodelling of the *M.tb* transcriptome during infection, highlighting the mechanisms of pathogenesis reflected therein.

## *M.tb* populations captured by *in vivo* transcriptional profiling

The considerable variation of parameters such as oxygen tension, pH or nutrient availability in the diverse intracellular and extracellular microenvironments encountered by *M.tb* during infection, together with stochastic host and pathogen interactions, likely results in a heterogeneous population of *in vivo* bacilli. This heterogeneity is reflected in the divergent transcriptional signatures of *in vivo*-derived *M.tb* [6-8]. The relative mRNA abundance measurements derived from gene expression analyses represent the average from the mixed populations of bacilli sampled. Furthermore, the experimentally determined transcriptional signature derived from a 70:30 combination of two phenotypically distinct populations (aerobically replicating:non-replicating persistence stage 2 bacilli) equates to the expected outcome using a simple additive model of gene expression [9]. The mechanistic insight or value for biomarker identification derived from the interpretation of these transcriptome datasets will therefore shift depending on the known bacterial heterogeneity in each scenario. Figure 1 summarises the broad and overlapping populations of bacilli that have been captured by gene expression profiling to date. The primary focus of research and the majority of mechanisms described in this review are based on observations derived from macrophage infection models due to the reasons described above, the relative low complexity of the system and because this is such a key step in mycobacterial pathogenesis.

The fundamental transcriptional adaptations that *M.tb* undergoes in an intracellular setting are summarised in Figure 2. While the exact environmental cues and regulatory cascades are not yet clearly defined, it is likely that many of these

systems are interdependent as the insults of infection drive overlapping bacterial responses.

## Lipid metabolism

*M.tb* is able to modulate its metabolism of carbon within the host phagosome, inducing genes involved in the β-oxidation of fatty acids and the catabolism of cholesterol. This shift to a lipolytic lifestyle is characterised by the up-regulation of multiple genes in the *fadA*, *fadB*, *echA*, *fadE*, and *fadD* (FACL) β-oxidation gene families; yielding acetyl-CoA and propionyl-CoA substrates (from even and odd chain number fatty acids respectively) for the TCA and methylcitrate cycles [2, 3, 10]. A cluster of genes (*Rv3492c-Rv3574*) implicated in the metabolism of cholesterol (to TCA intermediates) [11], many of which are regulated by *kstR1/kstR2* [12], are also activated intracellularly; as are key genes in the anaplerotic glyoxylate cycle and gluconeogenesis (*icl*, *pckA*). The induction of different genes in each class of β-oxidation enzyme has been observed after entry into human-derived [2] or murine-derived macrophages [3] or after macrophage-like THP-1 cell infection [13], suggesting that the action of these degradative enzymes may be specific to particular fatty acids present during infection. Interestingly, the magnitude of lipid metabolism gene regulation also varies with the changing phagocytic environment, with cholesterol and β-oxidation genes up-regulated to a greater extent after infection of non-permissive dendritic cells compared to macrophages [2]. Genes representative of gluconeogenesis, β-oxidation and glyoxylate cycles have also been identified to be induced in bacilli isolated from murine lung [8], from human granulomas [6, 7] and from sputum [9], and thus represent a key metabolic adaptation to intracellular and *in vivo* life.

The implications of switching to fatty acids as a carbon source are not fully understood. However, as lipid precursor pools and toxic intermediates fluctuate as carbon metabolism is transformed during infection, mechanisms are required to utilise or remove these moieties. For example, induction of the *M.tb* methylcitrate cycle inside macrophages may be a response to the accumulation of potentially toxic levels of CoA-propionate [10], such a build up of propionate might result from the metabolism of cholesterol [14]. This changing flux of propionate intracellularly also drives the synthesis of methyl-branched complex lipids such as phthiocerol dimycocersate which incorporate methyl-malonyl precursors derived from propionate [14, 15]. Genes involved in the biosynthesis of several complex lipids, postulated to be mediators of the immune system [16], are induced after macrophage infection [17, 18]. This raises the possibility that switching to an alternative carbon source in intracellular bacilli acts as a stimulus for, or directly results in, virulence factor expression.

## Carbohydrate metabolism

Carbohydrates are not hypothesised to be extensively metabolised during infection with pyruvate kinase (encoded by *pykA*), the major step in glycolysis and the metabolism of carbohydrates, repressed after monocyte-derived macrophage infection [2] and in sputum [9] relative to log phase axenic growth. The glycerol transporter encoded by *glpK* was also down-regulated intracellularly [2]. In contrast, the predicted carbohydrate transporters of unknown specificity (*sugI*, *Rv2040c* and *Rv2041c*) were induced on macrophage infection [2, 3], highlighting that the acquisition (or export) of carbohydrate moieties may still be important for successful

infection. Genes coding for the glycerol-3-phosphate transporter (*ugpA/B/E*) were also up-regulated after murine macrophage infection [3]. It has been suggested that carbohydrate (or amino acid) derived substrates are conserved for biosynthetic intent, while energy production is driven by fatty acid metabolism; reviewed in [10, 19]. The induction of *pckA*, encoding the first step in the biosynthesis of sugars from TCA-intermediates, intracellularly [2, 3, 13] and the essentiality of this gene for multiple stages of murine disease [20] infers that sugars are not widely accessible during infection.

## Nutrient acquisition

The mycobacterial phagosome has been hypothesised to be limited in nutrients required for bacterial growth [10, 21]. However is it difficult to interpret the transcriptional patterns derived from intracellular bacilli to define the complete nutritional status of the phagosome, with small numbers of differentially expressed genes identified from characterised operons, and limited overlap between infection models. Indeed without further investigation it is not clear whether the induction of genes encoding bidirectional amino acid metabolic enzymes reflects the presence (and opportunity to utilise) or absence (and therefore requirement to synthesise) of amino acid moieties. What is clear is that nutrient availability alters depending on the intracellular environment encountered, with genes involved in phosphate, arginine, histidine and tryptophan metabolism differentially expressed in bacilli isolated from human-derived macrophages compared to dendritic cells [2]. The induction of the stringent response regulator, *relA*, after dendritic cell relative to macrophage infection suggests that nutrient starvation may in part be responsible for the non-permissive nature of the dendritic cell microenvironment [2]. The stringent response,

mediated by *regX3*, may also be triggered by phosphate limitation in murine and guinea pig lung [22]. The differential regulation of genes involved in nutrient acquisition and the transportation of amino acids has also been demonstrated in the human lung [6]. See [21] for an excellent review of the physiology of mycobacteria.

The induction of genes coding for iron scavenging mycobactin and carboxy-mycobactin (gene clusters *mbtA-J* and *irtA/*B, *mbtL-N*) were observed after murine and human-derived macrophage infection [2, 3], and after murine macrophage infection with H37Rv or H37Ra *M.tb* bacilli [23]. However, the up-regulation of these genes was not consistently detected after infection of human-derived macrophage-like THP-1 cells [13], suggesting that the mycobacterial requirement for iron may shift depending on the phagosomal environment experienced. Correspondingly, genes encoding mycobactin were induced to a greater magnitude after IFNγ-activation of murine macrophages [3, 24], presumably reflecting a decrease in available iron intracellularly. Genes representative of mycobactin were also up-regulated in the murine lung, coinciding with the onset of the inflammatory immune response [4, 8]. Interestingly, *M.tb* bacilli may be able to utilise heme as a source of iron during infection, with *mhuD* (*Rv3592)* encoding a recently identified heme-degrading enzyme [25] induced after THP-1 infection [13]. Iron limitation may also act as a cue for virulence factor expression or carbon storage, with an increase in the abundance of triacylglycerols and the production of a novel wax ester observed in iron-limited bacilli [26]. Iron uptake systems were not induced in bacilli extracted from human lung sections [6] or from sputum [9]; indicating that iron sequestration may not be required at every stage of *M.tb* infection. For a review of iron uptake in mycobacteria see [27].

## Respiratory state

The plasticity of the *M.tb* respiratory chain throughout murine infection has been characterised by Shi and colleagues [28], who proposed that bacilli are able to alternate between three respiratory states in the transition from acute to chronic infection. During aerobic growth $aa_3$-type oxidase (*ctaBECD*), cytochrome C reductase (*qcrCAB*) and type I NADH dehydrogenase (*nuoA-N*) systems operate, as oxidative stress increases (or the environment becomes microaerophilic) bacilli switch to an intermediate state where the less-efficient cytochrome *bd* oxidase system (*cydCDBA*) is activated alongside nitrate reduction (*narK2*, *narGHJI*), finally respiration becomes anaerobic with nitrate utilised as a final electron acceptor. Definitive evidence for the role of nitrate in *M.tb* respiration remains elusive and it may be that the *narGHJI* gene cluster is functionally important in assimilation rather than reduction of nitrate as a terminal electron acceptor [29]. The shift to an intermediary microaerophilic respiratory state is observed in intracellular bacilli with the down-regulation of $aa_3$-type oxidase (*ctaACD*), cytochrome C reductase (*qcrCA*) and type I NADH dehydrogenase (*nuoA-N*) systems, and the induction of nitrate transporter (*narK2*) [2, 3]. Of note, the cytochrome *bd* oxidase system (*cydCDBA*) was demonstrated to be induced after human-derived macrophage infection [2], but repressed after murine macrophage infection [3]. Interestingly, genes involved in both aerobic and anaerobic respiration were expressed in human lung sections [6], likely reflecting the heterogeneous *in vivo* population of bacilli characterised. Transcriptional profiling of bacilli isolated from human sputa inferred the dominant respiratory signature to be microaerophilic or anaerobic [9].

The maintenance of redox potential in intracellular bacteria is likely to be markedly different to log phase axenic bacilli, with multiple studies observing the activation of genes involved in the response to oxidative/nitrosative stresses in bacilli extracted from macrophage [2, 3, 13, 24] or acute murine infection [4, 30]. The complexity of this reprogramming is underscored by the overlapping effects that multiple adaptations might have on the fluctuating redox status of intracellular and *in vivo* bacilli. For example, an increase in iron uptake through the induction of sequestration systems in intracellular bacilli may generate reactive oxygen intermediates [27], while the up-regulation of the cytochrome *bd* oxidase system may actively reduce intracellular oxidative stress [28].

## Immune mediator expression

Multiple mycobacterial factors have been identified that are able to modulate host immune responses [31] and many genes implicated in the synthesis and transport of these moieties are differentially regulated through the infection process. For example, *eis* (*Rv2416c*) encoding a secreted protein that may remodel cytokine secretion [32], is induced in both murine-derived macrophages [3] and in the murine lung [4]. The *esx* gene clusters encoding type VII secretion systems have also been demonstrated to be induced during murine macrophage infection [33] and in the murine lung [34]. It is likely that expression of these products, responsible for the export of antigenic proteins (exemplified by *esxA* and *esxB* coding for ESAT-6 and CFP-10) that are involved in membrane lysis [35], is carefully controlled by spatial and temporal determinants. Thus, *esx* genes have also been observed to be repressed after macrophage uptake [2, 3, 13]. Furthermore, representatives of this gene family were also differentially expressed between virulent (H37Rv) and

attenuated (H37Ra*) M.tb* strains after macrophage infection, highlighting the central role these effector molecules may play in pathogenicity [23]. The transcription of a second family of immunogenic proteins encoded by the PE/PPE genes, a subset of which are associated with ESX secretion systems, are also regulated by multiple environmental signals [36]. Many PE/PPE genes, with manifold hypothesised roles in mediating host-pathogen interactions, have been identified to be induced (11 [13]; 18 [2]; 21 [3]) or repressed (13 [13]; 10 [2]; 4 [3]) after macrophage infection. The differential regulation of these putative virulence factors mediated by diverse stimuli spotlights this family as an exciting prospect for future research.

Other transporters that may influence host-pathogen interplay are encoded by the *mce1-4* (mammalian cell entry) operons. These ABC uptake systems likely involved in the transportation of lipids, the abrogation of which dramatically affects virulence in mice and non-human primates (reviewed in [37]), are also differentially expressed after macrophage infection. The *mce1* (and *mce4*) operons were repressed after phagocytosis, whilst *mce2* and *mce3* operons were induced [2, 3]. The differential regulation of lipid transporters together with the induction of genes involved in the biosynthesis of *M.tb* complex lipids after macrophage infection [2, 17, 18] highlights a role for lipid factors in regulating host-pathogen interactions. These lipid moieties may regulate the hydrophobicity of the mycobacterial cell wall during infection, act as effector molecules modulating the host immune response, and/or be synthesised during intracellular growth to alleviate the toxic effects of fatty acid metabolism intermediates [10, 14, 15, 16, 38].

## Growth rate

Genes encoding *M.tb* ribosomal proteins (*rpl*, *rps* and *rpm* gene families) are repressed after macrophage infection in comparison to log phase axenic growth [2, 3]. This down-regulation of genes involved so intimately in microbial protein translation correlates with an initial slowing of *M.tb* multiplication after phagocyte infection. These changes are also accompanied by a repression of genes encoding ATP synthase subunits (*atpA-H*), which may reflect a changing energy requirement of intracellular bacilli. Furthermore, ribosomal genes are repressed with time after dendritic cell compared to macrophage infection; as the dendritic cell microenvironment becomes non-permissive for growth relative to the macrophage, where bacilli are able to replicate [2]. Ribosomal genes were also expressed at a lower level after IFNγ stimulation of murine macrophages correlating with an increasingly cidal intracellular niche [3]. This slow-growth signature has also been identified in bacilli extracted from sputum, alongside induction of genes involved in lipid metabolism [9]. Chemostat modelling of the transcriptomic changes associated with fast and slow mycobacterial growth revealed significant overlaps with gene expression patterns derived from intracellular bacilli; thus growth rate may influence the expression of multiple virulence genes and might therefore be classified as a virulence factor itself [39]. Mycobacterial growth is affected by numerous constraints and stresses, many of which are likely to fluctuate during intracellular and *in vivo* life, unravelling this relationship between physiological state, growth rate and virulence factor expression will expose key mycobacterial survival strategies.

## Regulation of gene expression

Thirteen sigma factors have been identified in *M.tb*, reviewed in [40], many of which have been demonstrated to be differentially regulated in the intracellular

environment, *sigA*, *sigB*, *sigC*, *sigD*, *sigE*, *sigG*, *sigH*, *sigJ*, *sigL* [2, 3, 18, 41], or after murine infection, *sigB*, *sigC*, *sigH*, *sigK* [4, 42]. The *M.tb* genome also includes 7 transcriptional regulators (*whiB1-7*) with homology to the *Streptomyces* WhiB sporulation factor. Of these, *whiB3* was up-regulated in the intracellular niche [13, 33], as was *whiB4* [24], *whiB6* [2] and *whiB7* [33]; while *whiB2* was down-regulated after macrophage infection [2, 13]. Additionally, the transcriptional regulators, *ethR*, *ideR*, *kstR* and *relA* have been identified as up-regulated intracellularly, and likely play important roles [2, 3, 12, 13].

*M.tb* utilises a number of the 11 two-component systems identified in the genome to sense and respond to the intracellular environment, *devRS* (*dosRS*), *kdpE*, *mprA*, *mtrA*, *phoP*, *prrA*, *regX3*, and *trcR* [2, 3, 13, 43]. Two of these regulatory systems, *phoPR* and *dosRST*, have been particularly well studied and exemplify the range of adaptations demanded of bacilli during infection. The *phoPR* two component system is required for virulence in macrophage and murine models of infection, regulating genes involved in complex lipid metabolism and ESX effector molecule expression; summarised in [44]. A point mutation in *phoP* may account for attenuation of H37Ra compared to H37Rv *M.tb* [45]. Moreover, comparison of the intracellular transcriptomes of H37Ra and H37Rv revealed differential regulation of these methyl-branched lipids and ESX-related genes, further highlighting the significance of this regulator in managing the macrophage environment [23]. Of additional importance to the site-specific expression of *phoP*-mediated virulence determinants, is the observation that the induction of *phoP*-regulated genes may be triggered by the slight acid environment (pH ~6.4) of the murine macrophage phagosome [33].

The *devRST* (*dosRST*) transcriptional regulator controls the expression of approximately 50 genes [46]. This regulon has been identified to be induced after macrophage infection [2, 3, 13, 33]; with an increase in magnitude of *dosR* regulon expression in IFNγ-activated murine macrophages dependent on a functioning murine *NOS2* gene [3], and over time after infection of non-permissive monocyte-derived dendritic cells relative to macrophages [2]. The *dosR* regulon was also up-regulated in bacilli isolated from artificial murine granulomas [42] and the murine lung following induction of the adaptive immune response [47]. Genes representative of the *dosR* regulon have also been identified to be induced in the human lung [7, 8], and a *dosR* signature is present in bacilli extracted from human sputa [9]. Therefore, genes regulated by *dosR* appear to be functionally significant in the adaption of *M.tb* bacilli to multiple stages of infection. Environmental stimuli that result in *dosR* induction are varied with hypoxia, nitric oxide, carbon monoxide, SDS, low pH and hydrogen peroxide implicated; reviewed recently [48]. It should be noted that although the *dosR* regulon is associated with a slowing of mycobacterial growth in many experimental models, this is not always the case with the *dosR* regulon induced in rapidly-replicating bacilli in a low-oxygen chemostat model (0.2% dissolved $O_2$ tension) [49]. The function of the *dosR*-mediated response remains to be fully elucidated; however it is probably important in the adaptation of *M.tb* to changes in redox status throughout the infectious process. Understanding the environmental cues that are important during infection, and the regulatory cascades that are triggered, will precipitate novel intervention strategies for combating *M.tuberculosis* disease.

## Conclusions

Insight into *M.tuberculosis* transcriptional responses that influence pathogenicity mostly comes from the comparison of intracellular or *in vivo* mycobacterial populations to log phase bacilli. This successful strategy, which enables transcriptional signatures to be easily contrasted, relies on a very well defined but ultimately contrived axenic *M.tb* growth state as a comparator condition. An alternative approach directly comparing *M.tb* intracellular transcriptomes after infection of different phagocytic cells [2], or after macrophage activation [3] reveals more subtle changes in gene expression as the microenvironment encountered by infecting bacilli diverges over time. Moreover studies contrasting the RNA signatures reflecting the fate of attenuated and virulent mycobacterial strains [23, 33], or the transcriptional modifications of bacilli *in vivo* after onset of the inflammatory immune response [4, 47] will begin to characterise what adaptations are necessary for bacilli to continue to survive the slings and arrows of outrageous infection. This work, aimed at exploring the changing physiological state of *M.tb* during disease, may also be used in conjunction with gene essentiality studies to identify tractable pathways for novel drug development targeting key *in vivo* populations of bacilli.

A major benefit of these global profiling datasets is the ability to attribute predicted action to genes of unknown function using gene regulatory or protein network modelling [24, 50]. Such interaction networks, together with metabolic models of *M.tb* [51], will be required to map and digest the huge quantity of mRNA abundance data generated from microarray and sequencing projects. Finally, *in vitro* transcriptional profiling of mycobacterial responses to selected stresses, metabolites and growth constraints in controlled settings will allow the multi-factorial signatures captured *in*

*vivo* to be dissected [3, 9, 12, 22, 26, 33, 39, 44, 49, 52, 53]. Thus, providing an interpretive framework of transcriptional patterns delineating complex *M.tb* responses and contributing to the development of *in vitro* models (applicable to whole cell screening) representing specific populations of bacilli that exist during natural infection.

Transcriptional profiling *M.tuberculosis* bacilli isolated from intracellular and *in vivo* infection settings has defined a set of core adaptations, such as the metabolism of lipids as a carbon source and shifting respiratory state, which are common to multiple (but most likely not all) stages of *M.tb* infection. As new technologies are developed for identifying, isolating and mRNA profiling distinct *M.tb* populations during infection [54], the contrasting characteristics of these bacilli and the niches they occupy will help to shape novel anti-mycobacterial drug and vaccine development.

## Acknowledgements

The author's research is funded by the European Commission 'New Medicines for TB - NM4TB' program (LHSP-CT-2005-018923). SJW would like to thank the Wellcome Trust for funding the Bacterial Microarray Group at St. George's (grant no. 062511), and Joanna Bacon and Philip Butcher for valuable comments on the manuscript.

The author declares no financial or commercial conflict of interest.

## Reference list


1. Cole, S.T., et al., *Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence.* Nature, 1998. **393**(6685): p. 537-44.
2. Tailleux, L., et al., *Probing Host Pathogen Cross-Talk by Transcriptional Profiling of Both Mycobacterium tuberculosis and Infected Human Dendritic Cells and Macrophages.* PLoS.ONE., 2008. **3**(1): p. e1403.
3. Schnappinger, D., et al., *Transcriptional Adaptation of Mycobacterium tuberculosis within Macrophages: Insights into the Phagosomal Environment.* J Exp Med, 2003. **198**(5): p. 693-704.
4. Talaat, A.M., et al., *The temporal expression profile of Mycobacterium tuberculosis infection in mice.* Proc.Natl.Acad.Sci.U.S.A, 2004. **101**(13): p. 4602-4607.
5. Waddell, S.J., et al., *Microarray analysis of defined Mycobacterium tuberculosis populations using RNA amplification strategies.* BMC Genomics, 2008. **9**(1): p. 94.
6. Rachman, H., et al., *Unique transcriptome signature of Mycobacterium tuberculosis in pulmonary tuberculosis.* Infect.Immun., 2006. **74**(2): p. 1233-1242.
7. Fenhalls, G., et al., *In situ detection of Mycobacterium tuberculosis transcripts in human lung granulomas reveals differential gene expression in necrotic lesions.* Infect.Immun., 2002. **70**(11): p. 6330-6338.
8. Timm, J., et al., *Differential expression of iron-, carbon-, and oxygen-responsive mycobacterial genes in the lungs of chronically infected mice and tuberculosis patients.* Proc Natl Acad Sci U S A, 2003. **100**(24): p. 14321-6.
9. Garton, N.J., et al., *Cytological and transcript analyses reveal fat and lazy persister-like bacilli in tuberculous sputum.* PLoS.Med., 2008. **5**(4): p. e75.
10. Munoz-Elias, E.J. and J.D. McKinney, *Carbon metabolism of intracellular bacteria.* Cell Microbiol, 2006. **8**(1): p. 10-22.
11. Van der Geize, R., et al., *A gene cluster encoding cholesterol catabolism in a soil actinomycete provides insight into Mycobacterium tuberculosis survival in macrophages.* Proc Natl Acad Sci U S A, 2007. **104**(6): p. 1947-52.
12. Kendall, S.L., et al., *A highly conserved transcriptional repressor controls a large regulon involved in lipid degradation in Mycobacterium smegmatis and Mycobacterium tuberculosis.* Mol.Microbiol., 2007. **65**(3): p. 684-699.
13. Fontan, P., et al., *Global transcriptional profile of Mycobacterium tuberculosis during THP-1 human macrophage infection.* Infect.Immun., 2008. **76**(2): p. 717-725.
14. Yang, X., et al., *Cholesterol metabolism increases the metabolic pool of propionate in Mycobacterium tuberculosis.* Biochemistry, 2009. **48**(18): p. 3819-21.
15. Minnikin, D.E., et al., *The methyl-branched fortifications of Mycobacterium tuberculosis.* Chem Biol, 2002. **9**(5): p. 545-53.
16. Cox, J.S., et al., *Complex lipid determines tissue-specific replication of Mycobacterium tuberculosis in mice.* Nature, 1999. **402**(6757): p. 79-83.
17. Li, M.S., et al., *cDNA-RNA subtractive hybridization reveals increased expression of mycocerosic acid synthase in intracellular Mycobacterium bovis BCG.* Microbiology, 2001. **147**(Pt 8): p. 2293-2305.
18. Graham, J.E. and J.E. Clark-Curtiss, *Identification of Mycobacterium tuberculosis RNAs synthesized in response to phagocytosis by human*

*macrophages by selective capture of transcribed sequences (SCOTS).* Proc Natl Acad Sci U S A, 1999. **96**(20): p. 11554-9.
19. Eisenreich, W., et al., *Carbon metabolism of intracellular bacterial pathogens and possible links to virulence.* Nat Rev Microbiol, 2010. **8**(6): p. 401-12.
20. Marrero, J., et al., *Gluconeogenic carbon flow of tricarboxylic acid cycle intermediates is critical for Mycobacterium tuberculosis to establish and maintain infection.* Proc Natl Acad Sci U S A, 2010. **107**(21): p. 9819-24.
21. Cook, G.M., et al., *Physiology of mycobacteria.* Adv Microb Physiol, 2009. **55**: p. 81-182, 318-9.
22. Rifat, D., W.R. Bishai, and P.C. Karakousis, *Phosphate depletion: a novel trigger for Mycobacterium tuberculosis persistence.* J.Infect.Dis., 2009. **200**(7): p. 1126-1135.
23. Li, A.H., et al., *Contrasting transcriptional responses of a virulent and an attenuated strain of Mycobacterium tuberculosis infecting macrophages.* PLoS One, 2010. **5**(6): p. e11066.
24. Rachman, H., et al., *Mycobacterium tuberculosis gene expression profiling within the context of protein networks.* Microbes.Infect., 2006. **8**(3): p. 747-757.
25. Chim, N., et al., *Unusual diheme conformation of the heme-degrading protein from Mycobacterium tuberculosis.* J Mol Biol, 2010. **395**(3): p. 595-608.
26. Bacon, J., et al., *Lipid composition and transcriptional response of Mycobacterium tuberculosis grown under iron-limitation in continuous culture: identification of a novel wax ester.* Microbiology, 2007. **153**(Pt 5): p. 1435-44.
27. Quadri, L.E.N., *Iron Uptake in Mycobacteria*, in *The Mycobacterial Cell Envelope*, M.a.R. Daffe, J. M., Editor. 2008, ASM Press: Washington DC. p. 167-184.
28. Shi, L., et al., *Changes in energy metabolism of Mycobacterium tuberculosis in mouse lung and under in vitro conditions affecting aerobic respiration.* Proc.Natl.Acad.Sci.U.S.A, 2005. **102**(43): p. 15629-15634.
29. Malm, S., et al., *The roles of the nitrate reductase NarGHJI, the nitrite reductase NirBD and the response regulator GlnR in nitrate assimilation of Mycobacterium tuberculosis.* Microbiology, 2009. **155**(Pt 4): p. 1332-9.
30. Shi, L., et al., *Transcriptional characterization of the antioxidant response of Mycobacterium tuberculosis in vivo and during adaptation to hypoxia in vitro.* Tuberculosis.(Edinb.), 2008. **88**(1): p. 1-6.
31. Pieters, J., *Mycobacterium tuberculosis and the macrophage: maintaining a balance.* Cell Host Microbe, 2008. **3**(6): p. 399-407.
32. Samuel, L.P., et al., *Expression, production and release of the Eis protein by Mycobacterium tuberculosis during infection of macrophages and its effect on cytokine secretion.* Microbiology, 2007. **153**(Pt 2): p. 529-40.
33. Rohde, K.H., R.B. Abramovitch, and D.G. Russell, *Mycobacterium tuberculosis invasion of macrophages: linking bacterial gene expression to environmental cues.* Cell Host.Microbe, 2007. **2**(5): p. 352-364.
34. Dubnau, E., et al., *responses of mycobacterium tuberculosis to growth in the mouse lung.* Infect Immun, 2005. **73**(6): p. 3754-7.
35. Simeone, R., D. Bottai, and R. Brosch, *ESX/type VII secretion systems and their role in host-pathogen interaction.* Curr Opin Microbiol, 2009. **12**(1): p. 4-10.
36. Voskuil, M.I., et al., *Regulation of the Mycobacterium tuberculosis PE/PPE genes.* Tuberculosis (Edinb), 2004. **84**(3-4): p. 256-62.

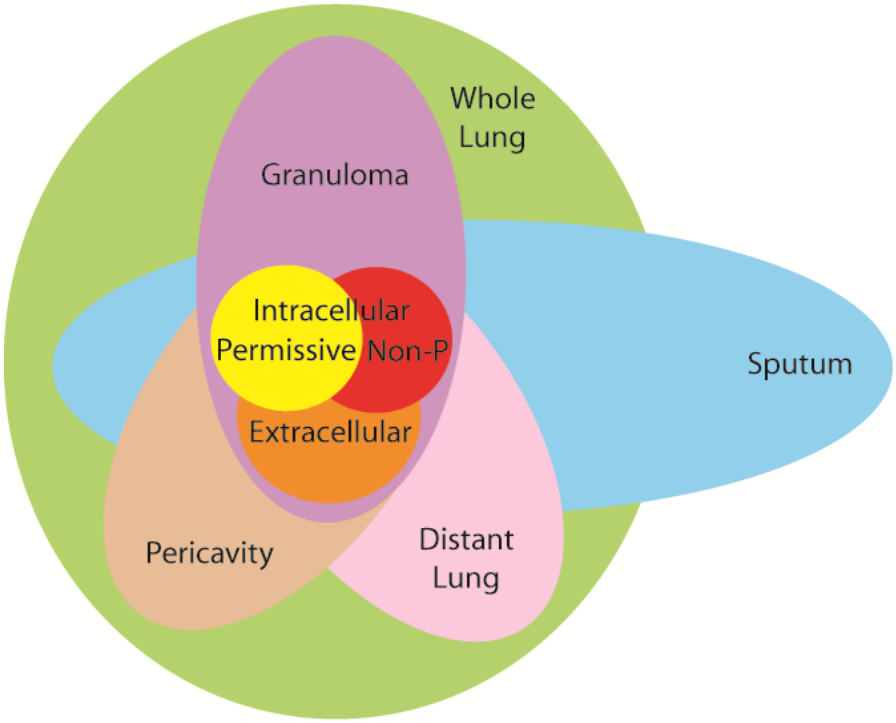
37. Casali, N. and L.W. Riley, *A phylogenomic analysis of the Actinomycetales mce operons.* BMC Genomics, 2007. **8**: p. 60.
38. Singh, A., et al., *Mycobacterium tuberculosis WhiB3 maintains redox homeostasis by regulating virulence lipid anabolism to modulate macrophage response.* PLoS.Pathog., 2009. **5**(8): p. e1000545.
39. Beste, D.J., et al., *Transcriptomic analysis identifies growth rate modulation as a component of the adaptation of mycobacteria to survival inside the macrophage.* J Bacteriol, 2007. **189**(11): p. 3969-76.
40. Rodrigue, S., et al., *The sigma factors of Mycobacterium tuberculosis.* FEMS Microbiol.Rev., 2006. **30**(6): p. 926-941.
41. Cappelli, G., et al., *Profiling of Mycobacterium tuberculosis gene expression during human macrophage infection: upregulation of the alternative sigma factor G, a group of transcriptional regulators, and proteins with unknown function.* Res Microbiol, 2006. **157**(5): p. 445-55.
42. Karakousis, P.C., et al., *Dormancy phenotype displayed by extracellular Mycobacterium tuberculosis within artificial granulomas in mice.* J.Exp.Med., 2004. **200**(5): p. 647-657.
43. Haydel, S.E. and J.E. Clark-Curtiss, *Global expression analysis of two-component system regulator genes during Mycobacterium tuberculosis growth in human macrophages.* FEMS Microbiol Lett, 2004. **236**(2): p. 341-7.
44. Gonzalo-Asensio, J., et al., *PhoP: a missing piece in the intricate puzzle of Mycobacterium tuberculosis virulence.* PLoS One, 2008. **3**(10): p. e3496.
45. Lee, J.S., et al., *Mutation in the transcriptional regulator PhoP contributes to avirulence of Mycobacterium tuberculosis H37Ra strain.* Cell Host Microbe, 2008. **3**(2): p. 97-103.
46. Park, H.D., et al., *Rv3133c / dosR is a transcription factor that mediates the hypoxic response of Mycobacterium tuberculosis* Mol.Microbiol., 2003. **48**(3): p. 833-843.
47. Shi, L., et al., *Expression of Th1-mediated immunity in mouse lungs induces a Mycobacterium tuberculosis transcription pattern characteristic of nonreplicating persistence.* Proc Natl Acad Sci U S A, 2003. **100**(1): p. 241-6.
48. Rustad, T.R., et al., *Hypoxia: a window into Mycobacterium tuberculosis latency.* Cell Microbiol, 2009. **11**(8): p. 1151-9.
49. Bacon, J., et al., *The influence of reduced oxygen availability on pathogenicity and gene expression in Mycobacterium tuberculosis.* Tuberculosis.(Edinb.), 2004. **84**(3-4): p. 205-217.
50. Balazsi, G., et al., *The temporal response of the Mycobacterium tuberculosis gene regulatory network during growth arrest.* Mol.Syst.Biol., 2008. **4**: p. 225.
51. Beste, D.J., et al., *GSMN-TB: a web-based genome-scale network model of Mycobacterium tuberculosis metabolism.* Genome Biol, 2007. **8**(5): p. R89.
52. Deb, C., et al., *A novel in vitro multiple-stress dormancy model for Mycobacterium tuberculosis generates a lipid-loaded, drug-tolerant, dormant pathogen.* PLoS One, 2009. **4**(6): p. e6077.
53. Betts, J.C., et al., *Evaluation of a nutrient starvation model of Mycobacterium tuberculosis persistence by gene and protein expression profiling.* Mol Microbiol, 2002. **43**(3): p. 717-31.
54. Barry, C.E., III, et al., *The spectrum of latent tuberculosis: rethinking the biology and intervention strategies.* Nat.Rev.Microbiol., 2009. **7**(12): p. 845-855.

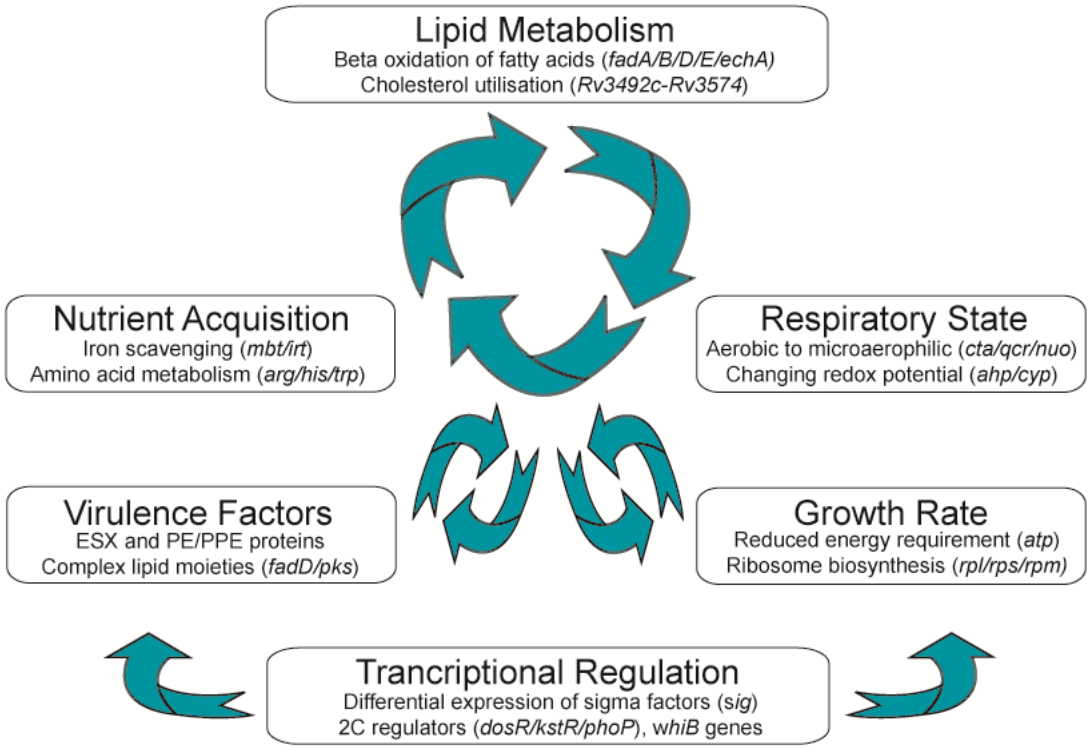
## Figure 1

The intracellular and *in vivo* populations of *M.tuberculosis* captured by transcriptional profiling. Each model examining the *in vivo* lifestyle of *M.tb* samples different populations of bacilli that may exist in diverse physiological states. Intracellular models of infection have characterised mycobacterial responses to environments both permissive and non-permissive for growth [2, 3]. Extracellular bacilli *in vivo* have not been profiled exclusively; however this population likely contributes to the gene expression patterns derived from selected regions of infected human lung [6] and from murine lung harvested during acute infection [4]. Sputa collected from patients may contain bacilli representative of all these populations together with bacilli adapted for successful transmission [9].

## Figure 2

The major themes summarising the reprogramming of the *M.tuberculosis* transcriptome in response to the intracellular or *in vivo* environments encountered during infection; based extensively on gene expression signatures derived from macrophage infection models. Representative gene families are marked in each class. The stimuli, signalling mechanisms and regulatory networks responsible for this remodelling require further elucidation. In addition, there is likely to be significant interplay between these transcriptional adaptations, influencing multiple facets of *M.tb* physiology throughout disease.


Whole
Lung
Granuloma
Intracellular
Permissive
Non-P
Extracellular
Sputum
Pericavity
Distant
Lung


Lipid Metabolism
Beta oxidation of fatty acids (fadA/B/D/E/echA)
Cholesterol utilisation (Rv3492c-Rv3574)
Nutrient Acquisition
Iron scavenging (mbt/irt)
Amino acid metabolism (arg/his/trp)
Respiratory State
Aerobic to microaerophilic (cta/qcr/nuo)
Changing redox potential (ahp/cyp)
Virulence Factors
ESX and PE/PPE proteins
Complex lipid moieties (fadD/pks)
Growth Rate
Reduced energy requirement (atp)
Ribosome biosynthesis (rpl/rps/rpm)
Trancriptional Regulation
Differential expression of sigma factors (sig)
2C regulators (dosR/kstR/phoP), whiB genes